Spinal Peroxynitrite Contributes to Remifentanil-Induced Postoperative Hyperalgesia via Enhancement of DMT1(-)IRE-Mediated Iron Accumulation in Rats

Authors: Ruichen Shu[1,2,],Linlin Zhang[1,2] , Chunyan Wang[1,2], Haiyun Wang[1,2], Nan Li[1,2], Guolin Wang[1,2,*]

[1] Department of Anesthesiology, Tianjin Medical University General Hospital, Tianjin 300052, China

[2] Tianjin Research Institute of Anesthesiology, Tianjin 300052, China

Background: Intraoperative analgesia using remifentanil is limited by the high incident of hyperalgesia. Peroxynitrite (PN) has been demonstrated to be a critical determinant in nociceptive process. Iron accumulation mediated by Divalent Metal Transporter 1 (DMT1), plays a key role in N-methyl-D-aspartate (NMDA) neurotoxicity, and it is an important interface between inflammatory and oxidative stress damage. This study aims to determine whether PN contributes to remifentanil-induced postoperative hyperalgesia via enhancement of DMT1-mediated iron accumulation.

Methods: Remifentanil and incision were involved in the rat model of remifentanil-induced postoperative hyperalgesia. Behavior testing was used to assess thermal and mechanical hyperalgesia. The expression of 3-nitrotyrosine (3-NT), nitrated manganese superoxide dismutase (MnSOD), DMT1(-)IRE and DMT1(+)IRE in protein of spinal cord were detected by immunoprecipitation and Western blot analysis. DMT1(-)IRE location in spinal section was examined with immunohistochemistry. Spinal iron concentration was measured using Perl's stain and atomic absorption spectrophotometer methods. Hydrogen-rich saline which imparts selectivity for PN decomposition and iron chelator (SIH) were applied in the mechanistic study on the roles of PN and iron, as well as prevention of hyperalgesia.

Results: Remifentanil induced thermal and mechanical hyperalgesia at postoperative 48 hours, and resulted in 3-NT formation and MnSOD nitration and inactivation. Increased expression of DMT1(-)IRE and iron accumulation were associated with remifentanil-induced postoperative hyperalgesia, while DMT1(+)IRE expression was unaffected. Iron chelation prevented nociceptive hypersensitivity in a dose-dependent manner. Eliminating PN with hydrogen-rich saline protected against hyperalgesia, and furthermore, it attenuated DMT1(-)IRE over-expression and iron accumulation.

Conclusions: Our study identifies that spinal PN activates DMT1(-)IRE, leading to abnormal iron accumulation in remifentanil-induced postoperative hyperalgesia, while providing the rationale for development of molecular hydrogen and "iron-targeted" therapies.

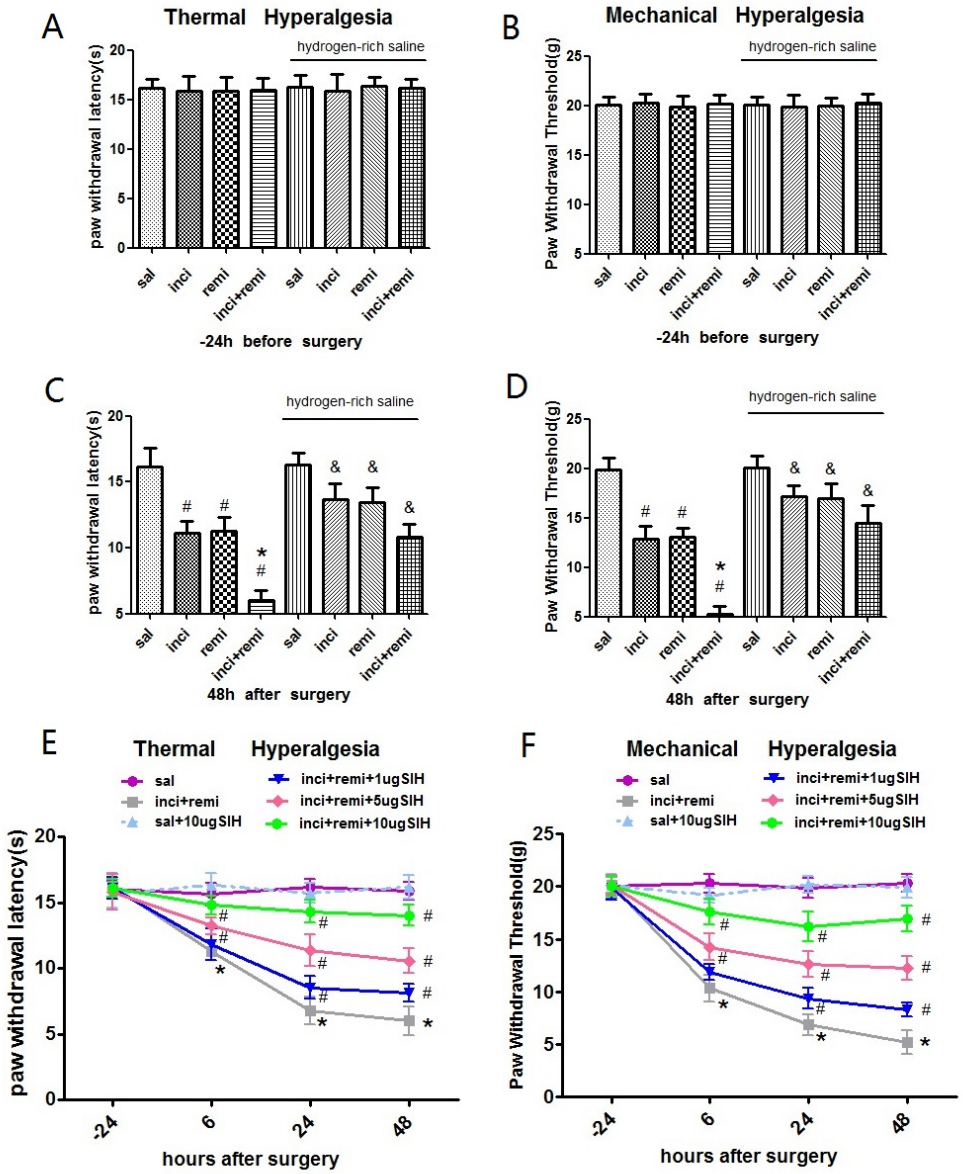

Fig. 1. **Hydrogen-rich saline (A-D) and iron chelator SIH (E-F) prevents remifentanil-induced postoperative hyperalgesia.** The baseline numbers of PWL (A) and PWT (B) were similar in all groups. When compared with rats receiving saline (sal), incision (inci) and remifentanil (remi) significantly increased PWL and PWT. Moreover, incision-remifentanil (inci-remi) treatment significantly enhanced hyperalgesia induced by incision. Intraperitoneal hydrogen-rich saline (10ml/kg) attenuated remifentanil-induced postoperative hyperalgesia. Results are expressed as mean ± SD for n=8 rats and analyzed by the two-way-repeated-measures ANOVA with Bonferroni comparisons. $^{\#}P$ <0.01 vs saline; *P <0.01 vs incision; $^{\&}P$ <0.01 vs corresponding non-hydrogen-rich-saline group.

When compared with vehicle (●), remifentanil (■) resulted in a time-dependent development of postoperative thermal (E) and mechanical (F) hyperalgesia. Intrathecal delivery of SIH (1ug,▼; 5ug,◆; 10ug,○) significantly attenuated the development of hyperalgesia in a dose-dependent manner (E, F). SIH in rats receiving saline (▲) had no effect on nociceptive thresholds. Results are expressed as mean ± SD for n=8 rats and analyzed by the two-way-repeated-measures ANOVA with Bonferroni comparisons. *P <0.01 for remifentanil versus vehicle and $^{\#}P$ <0.01 for remifentanil versus remifentanil+ SIH.

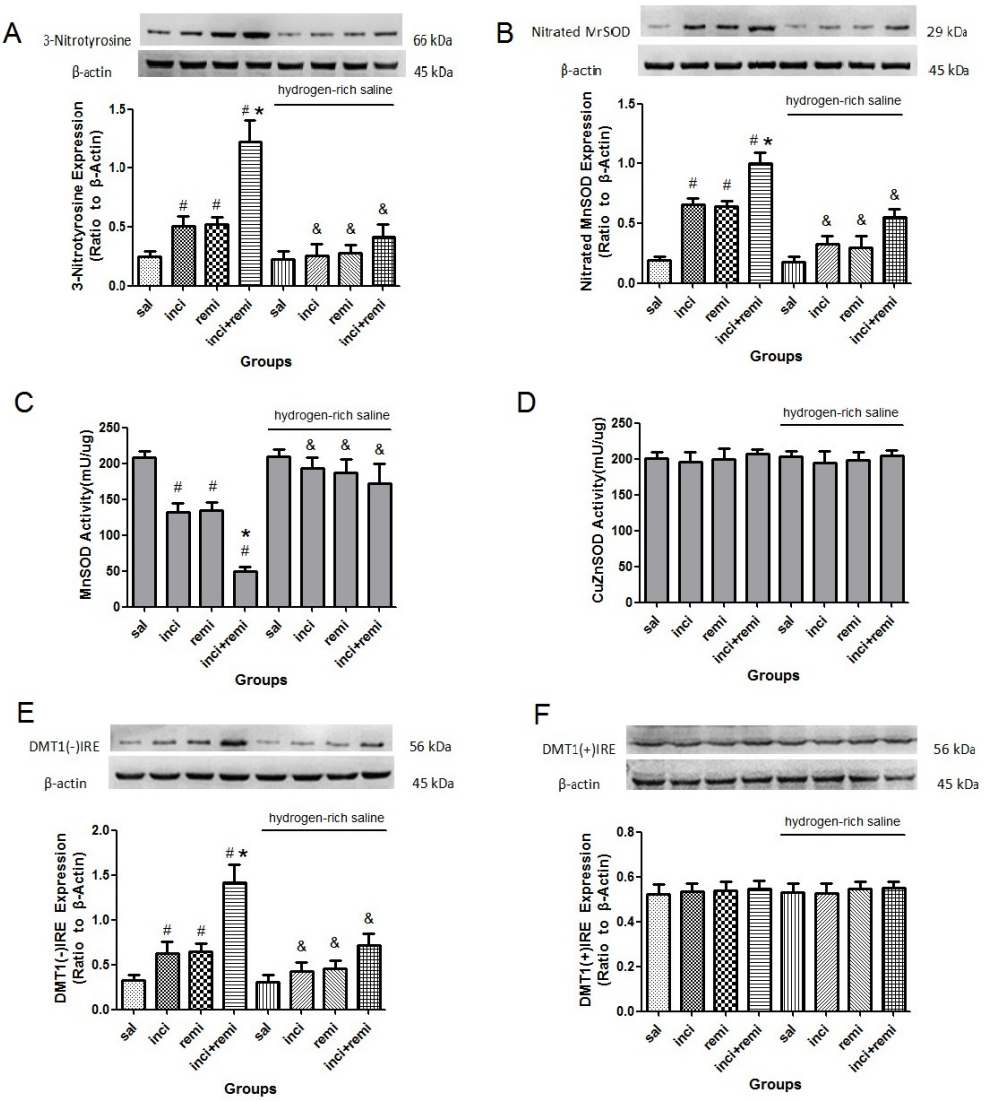

Fig. 2. **Increased PN formation, MnSOD nitration and inactivation and DMT1(-)IRE over-expression are associated with remifentanil-induced postoperative hyperalgesia, hydrogen-rich saline attenuates these abnormal changes.** 3-NT is a biomarker of PN. When compared with vehicle (sal), incision (inci) and remifentanil (remi) caused significant increase in 3-NT (A), nitrated MnSOD (B), DMT1(-)IRE (E) and decrease in MnSOD activity (C). Incision-remifentanil group (inci-remi) had greater levels of 3-NT, nitrated MnSOD and DMT1(-)IRE, less level of MnSOD activity than incision group. Intraperitoneal hydrogen-rich saline (10ml/kg) attenuated 3-NT formation (A), MnSOD nitration (B) and inactivation (C) and DMT1(-)IRE expression (E), it had no effect on rats receiving saline. Spinal CuZnSOD activity (D) and DMT1(+)IRE expression (F) was unchanged by any treatment. Results are expressed as mean ± SD for n=6 rats. Data are analyzed using the one-way ANOVA with Dunnett's post- hoc comparisons. $^{\#}P$ <0.01 vs saline; *P <0.01 vs incision; $^{\&}P$ <0.01 vs corresponding non-hydrogen-rich-saline group.

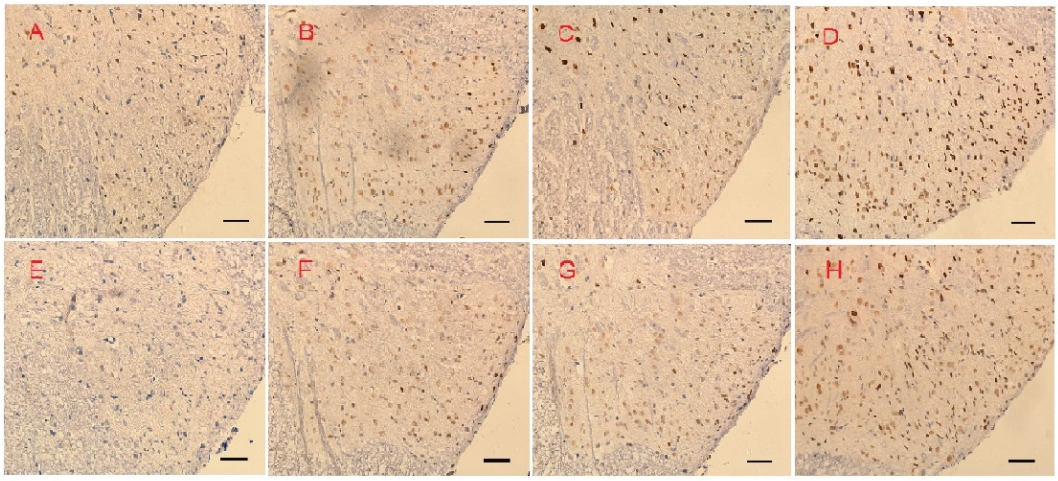

Fig. 3. **The spinal PN pathway is required for activation of DMT1(-)IRE.** Representative immunohistochemistry micrographs of dorsal horn of L4-L6 spinal cord showed that DMT1(-)IRE presents the brown staining and mainly locates at the nuclear of neurons. When compared with vehicle (A), the expression of DMT1(-)IRE slightly increased in rats receiving incision (B) and remifentanil (C) separately, and dramatically increased in incision-remifentanil rats (D). Intraperitoneal delivery of hydrogen-rich saline (10ml/kg) blocked the increasing DMT1(-)IRE expression respectively (F, G, H), but not its vehicle (E). Scale bar=50 μm.

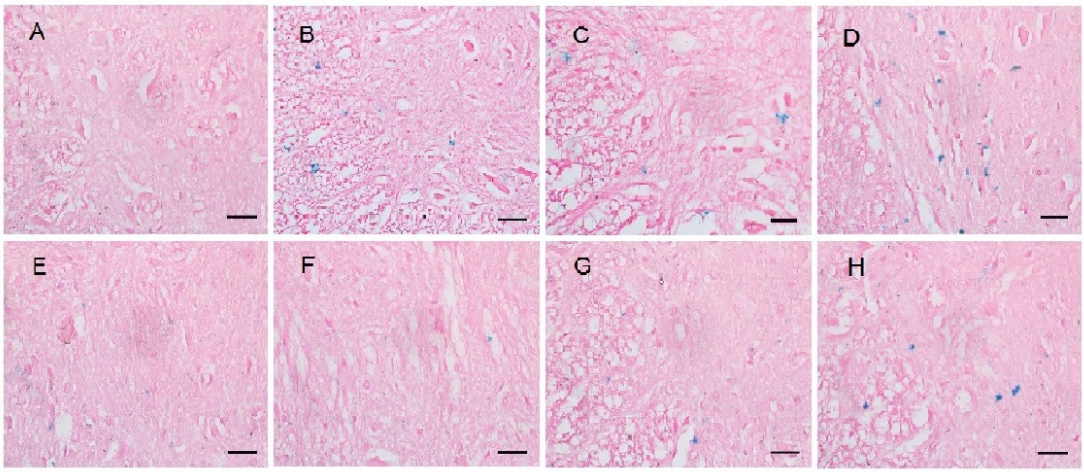

Fig. 4. **PN-activated DMT1(-)IRE over-expression leads to abnormal iron accumulation in remifentanil-induced postoperative hyperalgesia.** Iron accumulation in spinal cord were showed as blue deposits in Perl's stain micrographs. Hardly any iron accumulation was seen in the vehicle (A), there were mild iron accumulation in rats receiving incision (B) and remifentanil (C) separately, and pronounced iron accumulation in incision-remifentanil-treated rats (D). Intraperitoneal delivery of hydrogen-rich saline (10ml/kg) protected against abnormal iron accumulation respectively (F, G, H), but not its vehicle (E). Scale bar=50 μm.